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BIOTRANSFORMATION RATES OF THE BUTOXYETHYL ESTER OF 2,4-D BY BOTTOM AND SURFACE AUFWUCHS

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ABSTRACT

An experiment was conducted to investigate the variability in biotransformation rate coefficients of a xenobiotic, the butoxyethyl ester of 2,4-D, in natural waters between aufwuchs grown on Teflon strips located on the bottom and at the water surface of a pond and a river. The colonized strips and the natural waters were transported to the laboratory where the biotransformation studies were done under controlled conditions. Statistical analyses applied to the first-order rate coefficients showed a significant difference between bottom and surface aufwuchs for the river only. For both pond and river, a significant difference was shown when the aufwuchs was suspended, however. The aufwuchs mat thickness was significantly different between bottom and surface for the pond but not for the river and the biomass as ash-free dry weight was significantly different for both water bodies. The variability of biomass and first-order rate coefficients was higher with the bottom colonized aufwuchs than with the surface colonized aufwuchs.

INTRODUCTION

Biotransformation of xenobiotic compounds has been studied during the past ten years (Bartholomew and Pfaender 1983, Baughman et al. 1980, Larson 1980, Lewis and Holm 1981, Lewis et al. 1983, Paris et al. 1975, Paris et al. 1981, Paris et al. 1982, Paris et al. 1984, Parker and Doxtader 1982, Pritchard 1982, Simon-Sylvestre and Fournier 1979, Spain et al. 1980, Spain and Van Veld 1983, Steen et al. 1980, Van Veld and Spain 1983). Most of these studies concentrated on natural waters using suspended microorganisms and soil organisms. Few have studied biotransformation of xenobiotics in natural waters by aufwuchs communities (Lewis et al. 1983, Lewis and Holm 1981).

Most scientists agree that biotransformation studies should be done using mixed cultures of microorganisms collected from the natural environment (Bartholomew and Pfaender 1983, Baughman et al. 1980, Spain et al. 1980). Test systems and environmental conditions vary from study to study. A test system, certainly, should represent environmental conditions as closely as possible, otherwise too many unknowns are introduced, which increases the risk of not duplicating the actual environment. Field studies done entirely in the natural environment can escalate the cost prohibitively high and introduce much uncertainty. Finding, then, a way to do a field

study under semi-controlled conditions, that is a combination of field and laboratory, would appear to be an ideal solution.

Aufwuchs communities develop on most surfaces in natural aquatic systems and are usually sampled by colonizing substrates near water surfaces. Because of natural growth conditions, it would be safe to assume that aufwuchs structure changes with water depth or light intensity. The question arises as to what functional difference exists between these structurally different communities with respect to biotransformation of xenobiotics. To investigate these expected differences, the performance of aufwuchs communities grown uncontrolled on surfaces placed on the bottoms of a river and a pond was compared with that of communities grown at the water surface of the same natural systems, using the butoxyethyl ester of 2,4-dichlorophenoxy acetic acid (2,4-DBE) as the test compound in controlled laboratory studies. 2,4-DBE was selected for the study because of its rapid biodegradation and absence of an adaptation period (Lewis et al. 1983).

MATERIALS AND METHODS

Aufwuchs

Aufwuchs colonies that had been seasonallyexposed to temperatures between 2°C and 30°C were allowed to develop on Teflon strips (10 cm x 30 cm x 0.1 cm) that were situated on the bottoms and the water surfaces of a pond (surface area of 0.6 ha, maximum depth of 3 m) and a river (width of 15 m, flowrate of 8 m^3/sec), both located in the Athens, Georgia, area. The Teflon strips were suspended from a plastic-pipe frame held in place in the pond or river by either an anchor or steel pipes. Aufwuchs was allowed to colonize on the strips for 7 weeks in the river and 9 weeks in the pond to ensure mature transformation capacity (Lewis et al. 1985). The strips were then collected and transported to the laboratory in their natural water.

Kinetic Studies

Six strips were randomly selected from each of four sampling sites (river surface, river bottom, pond surface, and pond bottom). In the laboratory, replicate 4-L beakers for each site were set up, each containing three colonized strips and autoclaved natural water from its respective site which had been adjusted to a pH of between 6 and 7 with acid to prevent hydrolysis of 2,4-DBE. The aufwuchs surface (of three strips) area-to-volume ratio was 0.0204 m^2/L. Turbulence was achieved with stainless steel paddles driven by an electric motor at 63 rpm.

Rate studies were initiated by spiking with 2,4-DBE to an initial concentration of about 100 µg/L at a water temperature of 20°C and illumination of approximately 350 Lux. Duplicate samples were taken from each beaker at predetermined intervals, covering at least two half-lives of transformation. On day 2, the aufwuchs strips were transferred to a new set of beakers containing autoclaved natural water and the procedure of day 1 was repeated. On day 3, the biomass on the strips in each beaker was scraped off, blended for 5 sec at high speed, and poured into a new set of beakers containing autoclaved natural water. The procedures of the previous days were then repeated.

Biomass Determination

Separate sets of six colonized Teflon strips were retrieved from the bottom and the surface of the pond and from the bottom and the surface of the river, for the determination of aufwuchs mat thickness and ash-free dry weight (AFDW). Each of the 24 retrieved strips was weighed wet and again after drying for 2 hours at 105°C to determine the water content of the biomass. The dried biomass was then scraped off each strip and AFDW analyses were performed. The aufwuchs mat thickness was calculated by dividing the colonized Teflon strip area into the ratio of water content of biomass to density of water.

Analytical Methodologies

Concentrations of 2,4-DBE were determined by gas-liquid chromatography using a Tracor MT222 gas chromatograph equipped with a Ni63 electron capture detector and a 2-m column packed with 3% SE-30 on 80/100 mesh Chromosorb WHP, following extractions with 2,2,4-trimethyl pentane (iso-octane). 2,4-DBE (99.8% pure) was obtained from Union Carbide, Agricultural Products Division. The liquid was weighed into a volummetric flask and dissolved in methanol. A portion of this was transferred to an Erlenmeyer flask and the methanol was evaporated with dry nitrogen gas. Pure, filter-sterilized water was then added and stirred for 24 to 36 hours using a magnetic stirrer. Periodic analyses were performed to determine complete dissolution of the ester. From this stock, appropriate aliquots were added to test beakers to yield the desired test concentration.

Biomass determinations measured as AFDW were done according to Methods for Chemical Analysis (FPA 1979). Field water quality data were generated during the time of study using methods described in Methods for Chemical Analysis (EPA 1979).

Statistical Analysis

The t-Test was applied to determine significant differences at the 0.05 level between aufwuchs grown on the bottom and aufwuchs grown at the water surface. These calculations were done by using pooled data.

First-order biodegradation rate coefficients or slopes were calculated with linear least squares analysis using a first-order exponential decay expression:

$$C = Co\ e^{-Kt}$$

where

Co = initial concentration (µg/L)

C = concentration at time t

K = first-order rate coefficient or slope (min^{-1})

Results and Discussion

Teflon strips were colonized and transported to the laboratory where the rate studies were done in beakers under controlled conditions at a turbulence of 63 rpm. This value, being below the average linear flow of the rivers, prevented biomass from being dislodged from the surfaces. The turbulence in the pond test beakers was above the average low flow in the pond; however, biomass dislodged from these surfaces during day 1 was less than or equal to that dislodged from the surfaces in the river test beakers (about 10%).

Table 1. Aufwuchs mat thickness and ash-free dry weight values for pond after 8 weeks of colonization and for river after 6 weeks of colonization

	Pond		River	
	Mat Thickness µm	AFDW mg/cm^2	Mat Thickness µm	AFDW mg/cm^2
6 Surface Strips				
$\bar{X} \pm$ S.D.	278.5 ± 20.7	0.392 ± 0.052	111.2 ± 18.8	0.342 ± 0.079
CV	7.4%	13.2%	16.9%	23.1%
6 Bottom Strips				
$\bar{X} \pm$ S.D.	93.0 ± 50.1	0.201 ± 0.149	95.2 ± 34.9	0.219 ± 0.171
CV	53.9%	74.0%	36.6%	78.1%

The biomass data in Table 1, obtained using a separate set of Teflon strips, suggest a difference between pond and river biomass density for water surface colonized aufwuchs but not for bottom colonized aufwuchs, but this difference was not tested for statistically. The statistical evaluation testing for a difference between bottom and water surface colonized biomass was positive for both water bodies except for the aufwuchs mat thickness in the river (Table 2). This may be indicative of different populations growing on the bottom versus the surface in the pond but not in the river.

Significant differences detected in AFDW between bottom and water surface colonized biomass (Table 2) may be attributed to the effect of light diffusion to bottom and water surface aufwuchs resulting in variations in density and colonization. The higher variability in biomass measurements is an interesting finding as is the first-order rate coefficient of the bottom colonized aufwuchs versus the surface colonized aufwuchs for both water bodies.

Table 2. t-Test evaluation between bottom and water surface grown aufwuchs of first-order rate coefficients, aufwuchs mat thicknesses, and AFDW for pond and river

	Day 1	Day 2	Day 3	Biomass
Pond: K	N.S.	N.S.	S.	---
Mat Thickness	---	---	---	S.
AFDW	---	---	---	S.
River: K	S.	S.	S.	---
Mat Thickness	---	---	---	N.S.
AFDW	---	---	---	S.

K = first-order rate coefficient

S. = significant difference at 0.05 level

N.S. = no significant difference at 0.05 level

Table 3 shows the differences in water quality between the two waters. Both had low ionic strength and the river had a higher suspended particulate level.

The first-order biotransformation rate coefficients (Table 4) of day 2 can be considered a repeat of day 1. The values were somewhat higher on day 2 than on day 1, probably due to cell density increases during the 24-hr span. Rate coefficients for day 3 were obtained by suspending the aufwuchs and must be considered maximum transformation rate coefficients. A significant difference in transformation rate coefficients between bottom and surface colonized aufwuchs was found for the river during all three days, whereas a significant difference was found for the pond only during day 3 for suspended aufwuchs. The difference observed between pond and river on day 3 compared to day 1 and day 2 is a direct result of comparing intact versus suspended aufwuchs. Suspended aufwuchs exhibited negligible mass transport and/or diffusion limitations to the transformation of the ester. The diffusion limitation was most pronounced on day 1 and day 2, when the aufwuchs mat was intact. Intact river aufwuchs, having a higher biomass density, exhibited this limitation to a greater extent than the lower biomass density system of intact pond aufwuchs.

Table 3. Water chemistry data for pond and river during time of colonization

Parameter	Pond	River
NO_2-N (mg/L)	0.000	0.006
NO_3-N (mg/L)	0.007	0.608
NH_3-N (mg/L)	0.004	0.036
PO_4-P (mg/L)	0.006	0.059
Alkalinity (mg/L)	8.0	16.6
Hardness (mg/L)	6.0	14.3
Turbidity (FTU)	35	58
pH	6.6	6.9
Water temperature (°C)	27.5	25.4

Table 4. Means and standard deviations of first-order biodegradation rate coefficients (K*, $min.^{-1}$) for pond after 9 weeks of colonization and for river after 7 weeks of colonization

	Pond		
	Day 1	Day 2	Day 3
2 Surface Replicates			
K: $\bar{X} \pm$ S.D.	5.04 ± 0.50	6.08 ± 0.03	14.84 ± 2.60
2 Bottom Replicates			
K: $\bar{X} \pm$ S.D.	4.23 ± 1.71	4.26 ± 1.63	6.42 ± 2.28
	River		
	Day 1	Day 2	Day 3
2 Surface Replicates			
K: $\bar{X} \pm$ S.D.	9.58 ± 0.13	11.10 ± 0.41	18.40 ± 0.29
2 Bottom Replicates			
K: $\bar{X} \pm$ S.D.	3.35 ± 0.61	4.58 ± 0.46	5.76 ± 0.77

*rate coefficients multiplied by -10^3

Differences in biomass density between pond and river aufwuchs are attributed to differences in water flow characteristics and nutrient levels between the two water bodies. Those differences in water flow allowed pond aufwuchs to colonize filamentous microorganisms whereas those colonized on river surfaces under high flow conditions generally resulted in the colonization of tighter biomass mats.

Statistical analysis (t-Test) applied to the first-order rate coefficients showed a significant difference between bottom and surface aufwuchs for the river only. A significant difference for both, pond and river, was observed when the aufwuchs was suspended. The aufwuchs mat thickness between bottom and surface was significantly different for the pond but not for the river, and the biomass as ash-free dry weight was significantly different for both water bodies. First-order rate coefficients and biomass measurements indicated a higher variability with the bottom colonized aufwuchs than with the surface colonized aufwuchs.

In summary, a difference in biotransformation rate coefficients was observed in natural waters between bottom colonized aufwuchs and water surface colonized aufwuchs in a higher water

flowing system (river) but not in a nonturbulent water system (pond). Differences in biomass density between the river and pond systems and likewise limitations with respect to diffusion of chemical substrates within aufwuchs need to be researched further.

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